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Institute Report No. 338

Acute Oral Toxicity of Triethyleneglycol Dinitrate (TEGDN) in Sprague-Dawley Rats

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of triethyleneglycol dinitrate (TEGDN) was determined in male and female Sprague-Dawley rats by the oral gavage single dose method. The median lethal dose (MLD) was 1330 ± 42 mg/kg for male rats and 1116 ± 40 mg/kg for female rats. Clinical signs observed in this study included hunched posture, inactivity, tremors, twitching, and hypotonia. The duration of clinical signs was acute. Most animals were exhibiting signs by 4 hours after dosing and had either died or the signs had cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place TEGDN in the slightly toxic class.

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ABSTRACT

The acute oral toxicity of triethyleneglycol dinitrate (TEGDN) was determined in male and female Sprague-Dawley rats by the oral gavage single-dose method. The median lethal dose (MLD) was 1330 ± 42 mg/kg for male rats and 1116 ± 40 mg/kg for female rats. Clinical signs observed in this study included hunched posture, inactivity, tremors, twitching, and hypotonia. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or the signs had cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place TEGDN in the slightly toxic class.

KEY WORDS: Acute Oral Toxicity, Triethyleneglycol Dinitrate, TEGDN, Mammalian Toxicology, Propellant, Rat.

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

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US Army Biomedical Research and Development Command
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 84011

STUDY DIRECTOR: LTC Don W. Korte Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

CO-PRINCIPAL INVESTIGATORS: LTC Larry D. Brown, DVM, VC
Conrad R. Wheeler, PhD

PATHOLOGIST: MAJ John C. Turnier, DVM, VC, Diplomate
American College of Veterinary Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Triethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 24 January - 10 April 1985

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of triethyleneglycol dinitrate in Sprague-Dawley rats.

ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 John R.G. Ryabik, SP4 James J. Fischer, and SP4 Scott L. Schwebe provided research assistance and animal care; SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; Richard A. Spieler and Charlotte L. Speckman provided animal care and facility management; Colleen S. Kamiyama, Brenda V. Goce, and Steven Lau provided secretarial assistance. Michael J. Pearce, MS, organized the data, performed statistical analyses, and helped prepare the final report.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that study number 84011 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 1 June 89
DON W. KORTE JR, PhD / DATE
LTC, MSC
Study Director

Larry D. Brown 11 Jan 89
LARRY D. BROWN, DVM / DATE
LTC, VC
Co-Principal Investigator

for Don W. Korte Jr 1 June 89
GERALD F.S. HIATT, PhD / DATE
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Principal Investigator

Yvonne C Johnson 3 Feb 89
YVONNE C. JOHNSON, BS / DATE
DAC
Data Manager

Conrad Wheeler 3 Feb 89
CONRAD WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 26 June 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84011

1. This is to certify that is to certify that the protocol for LAIR GLP Study 84011 was reviewed on 24 February 1984.

2. The institute report entitled "Acute Oral Toxicity of Triethyleneglycol Dinitrate (TEGDN) in Sprague-Dawley Rats," Toxicology Series 133, was audited on 15 June 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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Acute Oral Toxicity of Triethyleneglycol Dinitrate (TEGDN) in Sprague-Dawley Rats--Hiatt *et al.*

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, a dermal toxicity test in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of triethyleneglycol dinitrate in male and female Sprague-Dawley rats.

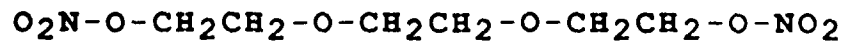
MATERIALS

Test Substance

Chemical Name: Triethyleneglycol dinitrate

Chemical Abstract Service Registry No.: 111-22-8

Chemical Structure:



$O_2N-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-NO_2$

Molecular Formula: $C_6H_{12}N_2O_8$

Source: Naval Ordnance Station, Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for TEGDN was corn oil (lot #13F-0705, Sigma Chemical Company, St. Louis, MO). The expiration date was December 1994.

Animal Data

Sprague-Dawley rats (Bantin & Kingman, Inc., Fremont, CA) from a shipment that arrived on 7 March 85 and was originally assigned to GLP protocol 84034 were used in this study. They were identified individually with ear tags. The animal weights on 7 March 85 ranged from 136 to 171 g. Study animals were transferred on 25 March 85 to GLP protocol 84011. Additional animal data appear in Appendix B.

Husbandry

Rats were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 23.1 to 26.4°C with a relative humidity range of 38 to 49 percent. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study rats were randomized into 5 dose groups of 10 males and 10 females. Allocation was accomplished using a computer-based stratified, weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. Seven animals, four males and three females, dosed with corn oil in an earlier phase of the study, served as vehicle controls. The animals were acclimated for 20 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 1000 and 1300 mg/kg. Based on these data, test doses were selected (Table 1).

Compound Preparation

TEGDN was received as a 10% solution in ethanol. The ethanol was removed with a rotary evaporator. TEGDN, a yellow brown oil, was then suspended in corn oil using a vortex mixer. The compound readily went into suspension and there was no discernible separation throughout the dosing procedures.

Chemical Analysis of Dosing Solution

NMR analysis demonstrated that the neat TEGDN is stable for at least one month (Appendix A). An emulsion of TEGDN in corn oil was stable for at least 24 hours. Tests for homogeneity of the test compound in the vehicle were conducted. The deviation of individual values from the mean of each set of 3 samples (top, middle, bottom) did not exceed 3.8% for any suspension.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3). Animals were fasted overnight before dosing. Volumes of the dosing suspension ranged from 0.93 to 1.30 ml in the males and 0.7 to 0.91 ml in females. The volumes given were based on 10 ml/kg body weight. The dose level was increased by varying the concentration of each suspension. Except for the highest female dose group, all dosing was performed on 27 March 85; the 1540 mg/kg female group was dosed on 4 April 85. The vehicle control group was given 0.6 ml corn oil on 7 February 85. The dosing was performed by oral gavage without animal sedation or anesthesia. Sterile disposable syringes (Becton, Dickenson & Co, Rutherford, NJ) fitted with 16-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized.

TABLE 1: TEGDN Doses

Group	Dose Level (mg/kg) Male	Female
3	931	867
4	1070	954
5	1240	1050
6	1430	1160
7	1650	1540
(vehicle)	0.6 ml corn oil	0.6 ml corn oil

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Observations were recorded at least twice on the day of dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 14-day study period were submitted for necropsy immediately after receiving a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD_{10}, LD_{50}, and LD_{90} were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete historical listing of study events.

Changes/Deviations

The study was accomplished according to the protocol and applicable amendments with the following exceptions: (a) three undosed females remaining after the study were transferred to another protocol; (b) due to misdosing, male groups 6 and 7 were reduced to 9 and 8 animals, respectively; and (c) animals were observed at least twice on the day after dosing rather than 1, 2 and 4 hours as stated in the protocol. These deviations did not significantly affect the outcome of the study.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Thirty-eight animals (39%) died as a result of the dosing. Fourteen deaths (37%) occurred within 12 hours of dosing. Twenty-three deaths (61%) occurred between 12 and 1[illegible] hours after dosing. The remaining animal died 72 hours after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2: Compound-Related Deaths by Group

Group	Dose Level (mg/kg)	Deaths/ Group	Percent Mortality
	MALE		
3	931	0/10	0
4	1070	1/10	10
5	1240	1/10	10
6	1430	7/9	78
7	1650	8/8	100
Vehicle	0	0/4	0
	FEMALES		
3	867	0/10	0
4	954	2/10	20
5	1050	4/10	40
6	1160	5/10	50
7	1540	10/10	100
Vehicle	0	0/3	0

Lethal Dose Calculations

Misdosed animals were excluded from statistical analysis and eliminated from the study. Lethal dose values were calculated by probit analysis and the equation for the probit regression line was: $Y = -58.2 \pm 20.2 \log X$ for males and $Y = -44.2 \pm 16.1 \log X$ for females, where X is the dose and Y the corresponding probit value. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

FIGURE 1

TEGDN Dose Response Curve in Male Sprague-Dawley Rats

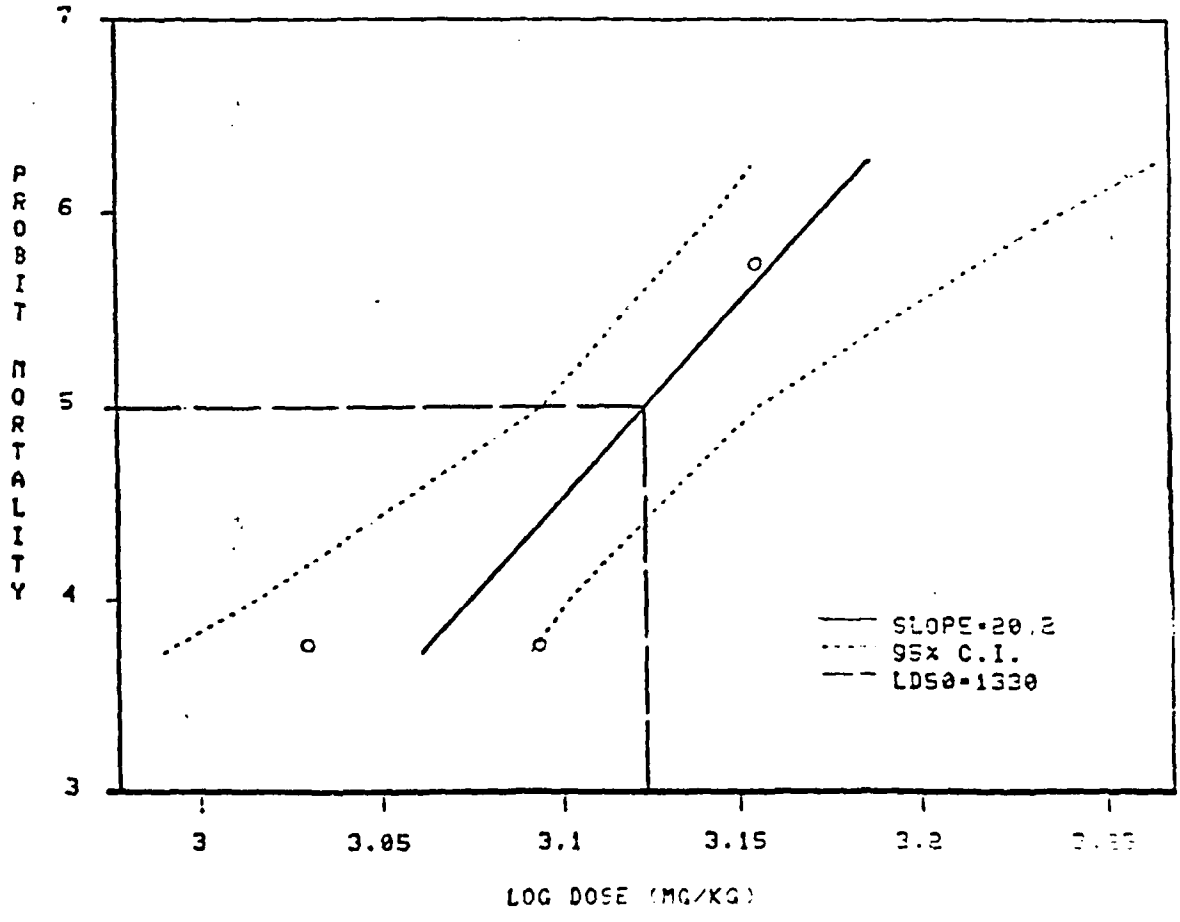


FIGURE 2

TEGDN Dose Response Curve in Female Sprague-Dawley Rats

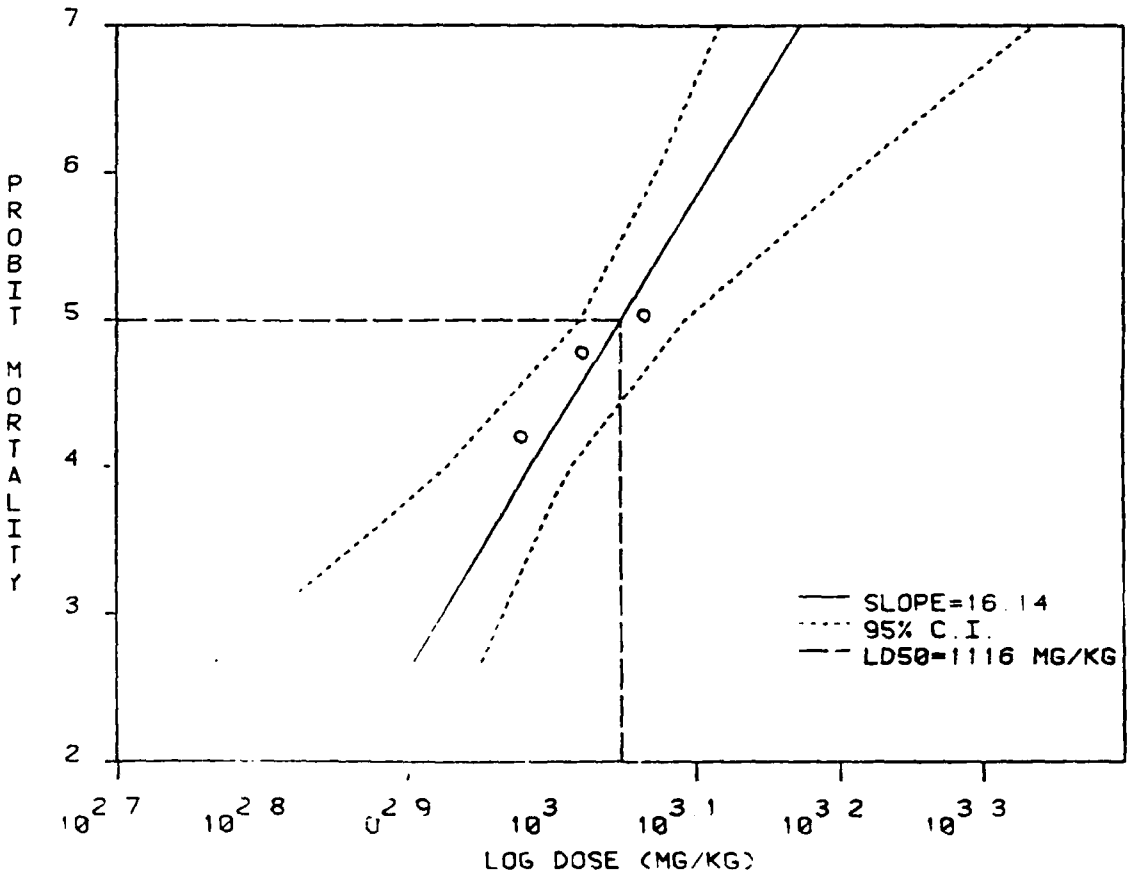


TABLE 3: Calculated Lethal Doses (LD) of TEGDN in Sprague-Dawley Rats

Percentage Lethality	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
	MALES	
LD_{10}	1150 ± 54	(977, 1233)
LD_{50}	1330 ± 42	(1243, 1432)
LD_{90}	1539 ± 78	(1430, 1841)
	FEMALES	
LD_{10}	930 ± 44	(785, 998)
LD_{50}	1116 ± 40	(1046, 1236)
LD_{90}	1340 ± 94	(1217, 1753)

* Calculated dose ± standard error.

Clinical Observations

The most frequently observed categories of clinical signs in animals administered TEGDN were behavioral disturbances (93 of 97 animals dosed), and hunched posture (86 of 97). Behavioral signs included irritability, inactivity, jumping, hyperactivity, hypotonia, tremors, and twitching. Oral and nasal trauma (17 of 97) was attributed to physical trauma associated with the more violent tremors and twitching that occurred in some animals. All animals that died exhibited one or more of the behavioral signs (except 3 animals died before signs could be recorded). Behavioral signs were present in all dosed groups and severity of response was dose related. Onset of these signs was usually before the initial observations 2 hours after dosing. In most surviving animals, these signs disappeared within 24 hours of dosing as the animals returned to normal.

Twenty-one female mice died during the study. Of the 29 surviving female mice, 15 were normal within 24 hours and another 3 mice were normal within 48 hours. Seventeen male mice died during the study. Of 30 surviving males, 18 were normal within 24 hours and another 7 were normal within 48

hours. All vehicle control animals survived until study termination at 14 days.

Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by administration of TEGDN. Table 5 presents the mean body weights by dose groups. Appendix F contains individual weight tables.

Gross Pathological Observations

Infarcts were present in the liver of one 1070 mg/kg male and the kidney of one 1160 mg/kg female. Hepatocellular vacuolization was present in the livers of one 1070 mg/kg and one 1650 mg/kg male as well as one 1050 mg/kg female and two 1540 mg/kg females. The above findings were not attributable to TEGDN administration. Hydronephrotic kidneys were considered incidental. The veterinary pathologist's report appears in Appendix G.

DISCUSSION

The calculated MLD for TEGDN was 1330 mg/kg in male rats and 1116 mg/kg in female rats. These values compare favorable with a reported oral MLD for TEGDN of 1000 mg/kg in male rats (5) and places TEGDN within the slightly toxic classification (6).

The acute lethality of TEGDN has been attributed to a combination of factors that include respiratory depression, tremors, and methemoglobin formation (7). Clinical signs associated with TEGDN toxicity were lethargy, tremors, and hyperreactivity to various stimuli. Signs of neurotoxicity were also observed in the present study as TEGDN produced a high incidence of tremors, twitching, and inactivity. The constant muscle activity invoked by TEGDN appeared to exhaust the animals and this could explain the inactivity and hypotonia reported. Another possible mechanism for the hypotonia is the observation that TEGDN interferes with cholinergic transmission at the neuromuscular junction.

Anderson *et al.* (7) reported a significant neural component to the toxicity profile of TEGDN characterized by tremors and hyperreactivity to stimuli as well as ataxia and lethargy. They attributed this TEGDN-induced neurotoxicity to the relatively long distance (10 atoms versus adjacent carbon atoms for PGDN) between nitrate groups which gives it

TABLE 4: Incidence Summary for Clinical Observations in Rats Administered TEGDN

Category of Clinical Signs	Group (N=)	Vehicle *	3 10	4 10	5 10	6 *	7 *
MALES	Dose (mg/kg)	Vehicle	931	1070	1240	1430	1650
Respiratory[a]		0	0	1	0	0	1
Behavorial[b]		2	10	10	10	8	8
Convulsions[c]		0	0	0	0	0	2
Gastrointestinal[d]		0	0	0	1	0	0
Hair[e]		0	1	0	0	0	0
Ocular[f]		0	0	1	3	3	2
Hunched Posture		0	10	10	10	6	6
Reflex[g]		0	1	2	1	1	0
Prostrate/Moribund		0	0	0	1	3	3
Miscellaneous[h]		0	3	2	4	4	4
Death		0	0	1	1	7	8
Normal		2	0	0	0	0	0
FEMALES	Dose (mg/kg)	Vehicle	867	954	1050	1160	1540
Respiratory[a]		0	0	0	0	0	0
Behavorial[b]		0	9	9	10	9	10
Convulsions[c]		0	1	0	0	0	0
Gastrointestinal[d]		0	0	0	0	0	0
Hair[e]		0	0	0	0	0	0
Ocular[f]		0	2	4	4	0	7
Hunched Posture		0	10	8	10	8	8
Reflex[g]		0	2	2	0	0	0
Prostrate/Moribund		0	0	1	1	3	5
Miscellaneous[h]		0	4	7	6	4	6
Death		0	0	2	4	5	10
Normal		3	0	0	0	0	0

*Vehicle control group had 3 females and 4 males. Group 6 had 10 females and 9 males. Group 7 had 10 females and 8 males.

[a]Includes changes in rate or depth.

[b]Includes jumping, irritability, inactivity, hyperactivity, hypotonia, tremors, and twitching.

[c]Includes clonic and tonic convulsions.

[d]Includes diarrhea.

[e]Includes alopecia.

[f]Includes squinting and lacrimation.

[g]Includes changes in the startle reflex.

[h]Includes urine stains on perineum and stains/material on mouth and nose.

TABLE 5: Mean Body Weights in Grams ± S.E (N)

Dose Groups (mg/kg)	At Receipt	Dosing Day	Midtrial Day	Termination Day
		MALES		
931	147.6 ±1.2 (10)	267.9 ±2.4 (10)	328.8 ±3.1 (10)	325.5 ±5.6 (10)
1070	144.4 ±1.4 (10)	275.1 ±4.6 (10)	335.7 ±7.6 (9)	333.7 ±7.1 (9)
1240	142.9 ±1.6 (10)	265.3 ±3.4 (10)	311.3 ±8.3 (9)	325.4 ±7.2 (9)
1430	145.7 ±1.7 (9)	267.0 ±3.9 (9)	337.5 ±21.5 (2)	318.5 ±16.3 (2)
1650	147.1 ±2.4 (8)	276.1 ±5.4 (8)	N/A	N/A
Vehicle	137.8 ±3.3 (4)	220.0 ±7.5 (4)	299.0 ±4.7 (4)	302.0 ±5.8 (4)
		FEMALES		
867	154.6 ±2.3 (10)	199.0 ±3.5 (10)	241.1 ±7.1 (10)	228.6 ±6.2 (10)
954	149.6 ±1.9 (10)	195.4 ±4.3 (10)	230.5 ±6.5 (8)	223.9 ±5.9 (8)
1050	151.4 ±0.7 (10)	192.6 ±1.9 (10)	230.8 ±3.9 (6)	222.3 ±4.0 (6)
1160	150.3 ±2.2 (10)	196.6 ±4.8 (10)	221.6 ±14.2 (5)	218.8 ±10.1 (5)
1540	151.9 ±1.3 (10)	208.9 ±4.7 (10)	N/A	N/A
Vehicle	144.3 ±1.4 (3)	182.3 ±1.9 (3)	223.7 ±6.1 (3)	215.7 ±2.3 (3)

decamethonium-like activity in addition to its nitrate ester actions. Thus, the tremors and twitching observed in this study following TEGDN administration could be attributable to a decamethonium-like action.

It is interesting that cyanosis was not observed following the TEGDN administration. Although this may be related to the difficulty in detecting cyanosis in rodents under artificial (fluorescent) light conditions such as are present in our animal facility, it more likely reflects the fact that TEGDN does not induce methemoglobin formation as readily as the classical nitrate esters. This is supported by Anderson *et al.* (7) who reported that at death, animals treated with TEGDN had 30-40% methemoglobinemia versus the 70-80% methemoglobinemia in animals treated with propyleneglycol dinitrate.

CONCLUSION

Triethyleneglycol dinitrate is a slightly toxic compound that produces signs of neurotoxicity in addition to standard symptoms of nitrate ester poisoning. Calculated MLD values were 1330 ± 42 mg/kg in male Sprague-Dawley rats and 1116 ± 40 mg/kg in female Sprague-Dawley rats.

REFERENCES

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Appendix A: CHEMICAL DATA

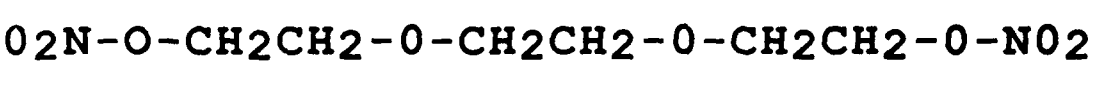
Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate, NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:



Molecular Formula: $C_6H_{12}N_2O_8$

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm^3): 1.32[1]

Manufacturer: Naval Ordnance Station
Indian Head, MD

Lot No.: 130-84

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. AD A127846, p. 17.

Appendix A (cont.): CHEMICAL DATA

Analytical Data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.[2] No impurities were detectable by NMR.[3] NMR (80 MHz, $CDCl_3$): 3.65 (S, 4H, $-CH_2-O-C\underline{H}_2C\underline{H}_2-O-CH_2-$), 3.72-3.84 (complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.[4]

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).[2] NMR analysis demonstrated that the neat compound is stable for at least 1 month.[3]

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[2] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p. 26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[4] Ibid. p. 64.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations

INTRODUCTION

Emulsions of triethyleneglycol dinitrate (TEGDN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP Studies #84010 (acute oral toxicity in mice) and #84011 (acute oral toxicity in rats). After dosing, the remainder of each emulsion was stored at 4°C for analysis. Determination of the TEGDN concentration was accomplished by reverse-phase liquid chromatography.

MATERIALS

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J.T. Baker Chemical Co., Phillipsburg, NJ.

METHODS

Analysis of TEGDN solutions was accomplished under the following HPLC conditions: solvent, 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injection volume, 10 µL; detector wavelength, 205 nm. The HPLC mobile phase was used to prepare standards as well as to extract the TEGDN/corn oil mixtures. Standards were prepared by weighing TEGDN on aluminium foil (0.5 mm squares) using a microbalance. The weigh boats containing TEGDN were added to volumetric flasks. The flasks were filled to volume and the contents mixed well by shaking. The concentration of the standards covering this ranged from 10.4 to 561.2 µg/ml and a set of standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

To extract the dosing preparations, the TEDGN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of five minutes an aliquot of approximately one ml was removed and transfered to a tared volumetric flask. The weight of each aliquot transfered was recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

To determine if the emulsions of TEGDN in corn oil prepared for dosing were homogenous, a series of emulsions was prepared with TEGDN concentrations spanning the range of concentrations employed in the dosing preparations. Four emulsions containing 50, 200, 400 and 800 mg of TEGDN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 min, aliquots from the top, middle, and bottom of the emulsions were removed and transfered to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transfered to a second volumetric flask for further dilution prior to HPLC analysis.

RESULTS

Under the conditions of the analysis, TEGDN eluted with a retention time of 4.4 min. A plot of the TEGDN concentration versus peak area was linear within the range of concentrations (10.4 to 561.2 µg/ml) employed as standards. Consecutive analyses (n = 10) were performed with standards containing 103.24, 301.84 and 608.2 µg TEGDN/ml. The coefficient of variation for each set of peak area values was 0.58%, 0.38%, and 0.27%, respectively. Standards were analyzed both before and after the analysis of samples prepared from dosing emulsions. The differences in peak areas between corresponding standards run before and after was less than 1%. As can be seen at the bottom of Tables 1 and 2, the standard plot was virtually identical from assay to assay.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in quantitative recovery of TEGDN with no peaks in the chromatogram from corn oil. The results for the determination of homogeneity are presented in Table 1. The deviation of individual values from the mean of each set of three samples (top, middle, bottom) did not exceed 3.8% for any emulsion prepared.

DISCUSSION

The quantitative recovery of TEGDN from corn oil emulsions, in addition to the linearity and reproducibility of the calibration plot over the period of the study, indicates the assay is a valid method to quantify TEGDN. The data in Table 1 demonstrates that the dispersion of TEGDN in corn oil provides a homogenous emulsion over a range of 50

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

to 800 mg/ml. Since the dosing preparations were prepared in an identical manner, they were, by implication, homogenous.

The data from the analysis of the dosing emulsions are presented in Table 2. For several samples the concentration of TEGDN determined by analysis showed significant deviation from the target concentration. Of the thirteen emulsions analyzed three showed deviations of greater than 10% from the target concentration. Reanalysis of two of these samples yielded results within three percent of the original values, thus confirming the initial results.

CALCULATIONS

A series of standards were analyzed before and after the samples (diluted dosing emulsions) for each study. The two peak area values for each standard solution were averaged and linear least-squares regression performed on the concentration versus peak area data. This provided the equation for the best fitting line in the form of Equation 1 in which:

Equation 1 $$Y \text{ (peak area)} = mX + b$$

m is the slope, X is the concentration (µg/ml) and b is the intercept. The concentration of TEGDN in the final dilution was calculated by substituting for Y the peak area obtained from HPLC analysis and solving for X.

The total amount of TEGDN in the sample analyzed was then calculated as shown in Equation 2.

Equation 2 $$\text{Total TEGDN (mg)} = \frac{X \times (\text{dilution factor})}{10^3 \text{ µg/mg}} = Y$$

The volume corresponding to the weight of TEGDN calculated above was determined by dividing by the density of TEGDN (Equation 3).

Equation 3 $$\text{Total TEGDN (ml)} = \frac{\text{Total TEGDN (mg)}}{1320 \text{ mg/ml}} = \frac{Y}{1320} = Z$$

The contribution by corn oil to the volume of the original aliquot of emulsion removed for analysis was calculated as follows:

Equation 4

$$\text{Volume of corn oil} = \frac{\text{Weight of aliquot removed for analysis} - \text{TEGDN}}{\text{Density of corn oil}} = \frac{\text{aliquot} - Y}{0.918\ \text{g/ml}} = V$$

The concentration of TEGDN could then be determined as follows:

Equation 5

$$\text{Conc. of TEGDN (mg/ml)} = \frac{\text{mg TEGDN}}{\text{total volume of aliquot removed for analysis}} = \frac{\text{mg TEGDN}}{\text{ml TEGDN + corn oil}} = \frac{Y}{Z + V}$$

REFERENCE

Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, p. 1-27. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

TABLE 1

Assessment of homogeneity for TEGDN/corn oil emulsions. Aliquots of approximately 1 ml were withdrawn from the top (T), middle (M), and bottom (B) of the emulsions prepared to represent the range of TEGDN concentrations [TEGDN] employed in dosing. Three samples were analyzed from the top layer to determine the within-layer variability in the sampling and analysis.

Target [TEGDN] (mg/ml)	Site of sampling	[TEGDN] (mg/ml) determined by analysis	Mean [TEGDN] (mg/ml) of top samples $(T+T+T)/3=X_T$	Mean [TEGDN] (mg/ml) of all samples $(X_T + M + B)/3$	Deviation from mean (%)
	T	45.8			
	T	47.4	47.0	47.3	-0.6%
50*	T	47.7			
	M	48.2			+1.9%
	B	46.8			-1.1%
	T	205.2			
	T	209.6	207.9	210.4	-1.2%
200†	T	209.0			
	M	211.4			+1.5%
	B	212.0			+0.8%
	T	426.0			
	T	418.8	421.0	423.4	-0.6%
400‡	T	418.3			
	M	419.4			-0.9%
	B	429.7			+1.5%
	T	805.7			
	T	824.7	801.6	833.3	-3.8%
800§	T	774.3			
	M	844.6			+1.4%
	B	853.6			+2.4%

*Equation for the standard curve: $Y = 0.0453X + 0.2426$.
†Equation for the standard curve: $Y = 0.0465X - 0.0622$.
‡Equation for the standard curve: $Y = 0.0459X + 0.0639$.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

TABLE 2

Concentration of TEGDN [TEGDN] in dosing emulsions prepared for GLP Studies 84010 and 84011. Samples that were analyzed a second time for verification have been denoted with a R (reanalyzed) in front of the target concentration. In each case reanalysis yielded a value for concentration that was within 3% of the initial determination.

Study Number	Target [TEGDN] (mg/ml)	Date Prepared	Date Analyzed*	[TEGDN] determined by Analysis (mg/ml)	% of Target [TEGDN]
	215.0	8 Apr 85	10 Jun 85	237.8	110.6
	245.0	8 Apr 85	10 Jun 85	257.3	105.0
	278.0	8 Apr 85	10 Jun 85	283.2	101.9
84010	316.0	8 Apr 85	10 Jun 85	334.1	105.7
	167.0	9 Apr 85	1 Jul 85	169.4	101.4
	360.0	9 Apr 85	10 Jun 85	392.6	109.1
	129.0	12 Apr 85	1 Jul 85	133.3	103.3

Study Number	Target [TEGDN] (mg/ml)	Date Prepared	Date Analyzed*	[TEGDN] Determined by Analysis (mg/ml)	% of Target [TEGDN]
	212.0	26 Mar 85	14 Aug 85	227.5	107.3
	234.0	26 Mar 85	14 Aug 85	210.6	90.0
	R 257.0	26 Mar 85	14 Aug 85	329.1	128.1
84011	285.0	26 Mar 85	14 Aug 85	284.1	99.7
	R 378.0	26 Mar 85	14 Aug 85	417.2	110.4
	428.0	4 Apr 85	14 Aug 85	461.6	107.9

*The correlation coefficient and equation of the line for the standard plot on each date of analysis is given as follows:

Date of Analysis	Correlation Coefficient	Equation
10 Jun 85	0.9999	y = 0.0454 X + 0.0877
1 Jul 85	0.9999	y = 0.0455 X + 0.0527
28 Jul 85	0.9999	y = 0.0453 X + 0.0782
17 Jul 85	0.9999	y = 0.0454 X + 0.0385
25 Jun 85	0.9999	y = 0.0454 X + 0.0746
14 Aug 85	0.9999	y = 0.0461 X + 0.0838

Appendix B: ANIMAL DATA

Species: *Rattus norvegicus*

Strain: Sprague-Dawley

Source: Bantin and Kingman, Inc
Fremont, CA 94538

Sex: Male and female.

Date of birth: Males TEGDN : 28 Jan 1985
Vehicle control: 17 Dec 1984
Females TEGDN: 23 Jan 1985
Vehicle control 10 Dec 1984

Method of randomization: Weight bias, stratified animal allocation (TOXSYS® Animal Allocation Program)
SOP OP-ISG-24

Animals in each group: 10 males and 10 females - Groups 3-7
4 males and 3 females - vehicle control

Condition of animals at start of study: Normal

Body weight range at dosing: 171-297 g

Identification procedures: Ear tag

Pretest conditioning: Quarantine/acclimation 7-27 March 85 (20 days) for males and females.

Justification: The laboratory rat has proven to be a sensitive and reliable animal model for lethal dose determinations.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
24 Jan 85	Rats used as vehicle controls for GLP Protocol 84011 were received. Rats were checked for condition, sexed, ear-tagged, weighed, and individually caged.
25 Jan - 6 Feb 85	Vehicle controls were observed.
31 Jan 85	Vehicle controls were weighed.
7 Feb 85	Vehicle controls were weighed, dosed, and observed for signs of toxicity.
8-21 Feb 85	Vehicle controls were observed daily in a.m. and p.m.
14 Feb 85	Vehicle controls were weighed.
21 Feb 85	Vehicle controls were fasted, weighed, and submitted for necropsy.
7 Mar 85	Rats were received for GLP protocol 84034 and checked for condition, sexed, weighed, ear-tagged, and individually caged.
8-26 Mar 85	Animals were observed daily.
13 Mar 85	Four rats (2 male and 2 female) were submitted for quality control necropsy.
13,21 Mar 85	Animals were weighed.
21 Mar 85	Animals were randomized into dose groups.
25 Mar 85	Animals were transferred from GLP protocol 84034 to GLP protocol 84011.
27 Mar 85	Animals were weighed and male groups 3-7 and female groups 3-6 were dosed at approximately 1000 hours. Observations were conducted at two and four hours after dosing.
28 Mar - 9 Apr 85	Animals were observed twice daily, once in the a.m. and once in the p.m.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

Date	Event
3,10 Apr 85	Animals were weighed.
4 Apr 85	Group 7 females were weighed and dosed at approximately 1130 hours. Observations were conducted at two and four hours after dosing.
1[illegible] Apr 85	Animals were weighed and submitted for necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing									
		Hours		Days							
		4	12	1	2	3	4	5	6	7	8-14
				MALES							
931	10	0	0	0	0	0	0	0	0	0	0
1070	10	0	1	1	1	1	1	1	1	1	1
1240	10	0	0	1	1	1	1	1	1	1	1
1430	9	1	3	6	7	7	7	7	7	7	7
1650	8	1	3	6	8	8	8	8	8	8	8
Vehicle	4	0	0	0	0	0	0	0	0	0	0
				FEMALES							
867	10	0	0	0	0	0	0	0	0	0	0
954	10	1	1	1	2	2	2	2	2	2	2
1050	10	0	0	1	3	3	4	4	4	4	4
1160	10	2	4	5	5	5	5	5	5	5	5
1540	10	0	2	8	10	10	10	10	10	10	10
Vehicle	3	0	0	0	0	0	0	0	0	0	0
TOTAL		5	14	29	37	37	38	38	38	38	38

Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALE: 931 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00216	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
85D00210	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00206	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
85D00203	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
85D00192	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27-29	Marked
	Hypotonia	Mar 27-29	Marked
	Tremors	Mar 27	Slight
	Twitching	Mar 27	Slight
85D00189	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27,28	Moderate
	Hypotonia	Mar 27,28	Moderate
	Stain, Mouth, Brown	Mar 27	Slight
85D00178	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00176	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Inc. Startle Reflex	Mar 27	Slight
	Material, Mouth, Clear	Mar 27	Moderate
	Material, Perianal, Yellow	Mar 27	Slight
	Alopecia, R. Abdomen	Apr 7,10	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 931 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00163	Hunched Posture	Mar 27,28	Slight
	Irritable	Mar 27,28	Slight
	Stain, Nose, Brown	Mar 27	Slight
85D00160	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate
	Irritable	Mar 28	Slight
	Tremors	Mar 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1070 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00215	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
85D00214	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Marked
85D00213	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00205	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
	Death	Mar 27	5.6 h
85D00204	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
	Material, Nose, Red	Mar 27	Moderate
	Squinting	Mar 27	Slight
	Irritable	Mar 28	Slight
85D00196	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Moderate
85D00193	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Hypertonia	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate
	Tremors	Mar 27	Moderate
85D00184	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1070 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00175	Tachypnea	Mar 27	Slight
	Inactive	Mar 27	Slight
	Hunched Posture	Mar 27	Marked
	Inc. Startle Reflex	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00170	Tremors	Mar 27	Moderate
	Inc. Startle Reflex	Mar 27	Slight
	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Material, Nose, Brown	Mar 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1240 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00211	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate
85D00209	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Tremors	Mar 27	Slight
	Hypotonia	Mar 27	Slight
	Material, Mouth, Red	Mar 27	Slight
	Irritable	Apr 6	Slight
	Diarrhea	Apr 7,10	Slight
85D00188	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Slight
	Squinting	Mar 27	Slight
	Material, Perianal, Yellow	Mar 28	Slight
85D00186	Hunched Posture	Mar 27-31, Apr 3,4	Marked
	Inactive	Mar 27-30, Apr 4	Marked
	Hypotonia	Mar 27-31, Apr 3	Marked
	Twitching	Mar 27	Marked
	Squinting	Mar 27	Slight
	Material, Perianal, Brown	Mar 28-31	Marked
	Material, Mouth, Red	Mar 28	Slight
85D00180	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Twitching	Mar 27	Slight
	Hypotonia	Mar 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1240 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00174	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Marked
	Stain, Nose, Brown	Mar 27	Slight
85D00171	Hunched Posture	Mar 27	Slight
	Jumping	Mar 27	Slight
	Inactive	Mar 27	Slight
	Inc. Startle Reflex	Mar 27	Moderate
	Squinting	Mar 27	Slight
	Tremors	Mar 27	Marked
	Prostrate	Mar 27	Present
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Death	Mar 28	20.4 h
85D00168	Inactive	Mar 27	Moderate
	Hunched Posture	Mar 27	Marked
	Hypotonia	Mar 27	Moderate
85D00162	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
	Irritable	Mar 27	Slight
85D00161	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Tremors	Mar 27	Slight
	Hypotonia	Mar 27	Moderate
	Irritable	Mar 28	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1430 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00220	Death	Mar 27	3.0 h
85D00219	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27,28	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27,28	Marked
	Material, Mouth,Brown,Red	Mar 27-29	Slight
	Material, Perianal, Yellow	Mar 28,29	Moderate
	Death	Mar 29	2.1 days
85D00207	Prostrate	Mar 27	Present
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
	Death	Mar 27	4.5 h
85D00202	Hunched Posture	Mar 27	Marked
	Tremors	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Prostrate	Mar 27	Present
	Twitching	Mar 27	Moderate
	Material, Mouth, Red	Mar 27	Moderate
	Death	Mar 28	19.0 h
85D00198	Hunched Posture	Mar 27	Marked
	Tremors	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Material, Perianal, Clear	Mar 27	Moderate
	Twitching	Mar 27	Slight
	Squinting	Mar 27	Slight
	Death	Mar 28	19.0 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1430 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00182	Hunched Posture	Mar 27	Marked
	Jumping	Mar 27	Marked
	Inc. Startle Reflex	Mar 27	Moderate
	Squinting	Mar 27	Slight
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Death	Mar 28	19.0 h
85D00179	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27-29	Marked
	Hypotonia	Mar 27-30	Slight
	Irritable	Mar 27,30-31	Slight
85D00172	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00166	Tremors	Mar 27	Marked
	Twitching	Mar 27	Marked
	Jumping	Mar 27	Moderate
	Squinting	Mar 27	Moderate
	Prostrate	Mar 27	Present
	Hypotonia	Mar 27	Marked
	Material, Mouth, Red	Mar 27	Moderate
	Death	Mar 27	4.6 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1650 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00218	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Death	Mar 27	4.2 h
85D00201	Clonic Convulsion	Mar 27	Present
	Tonic Convulsion	Mar 27	Present
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Inactive	Mar 27	Marked
	Death	Mar 27	4.2 h
85D00200	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Prostrate	Mar 27	Present
	Twitching	Mar 27	Moderate
	Death	Mar 27	18.8 h
85D00199	Hunched Posture	Mar 27-29	Marked
	Inactive	Mar 27-29	Marked
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27-29	Marked
	Twitching	Mar 27	Slight
	Material, Perianal, Yellow	Mar 28,29	Slight
	Material, Mouth, Clear	Mar 28	Moderate
	Material, Eye, Yellow	Mar 29	Moderate
	Inc. Respiratory Depth	Mar 29	Moderate
	Death	Mar 29	1.8 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1650 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00190	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27,28	Moderate
	Material, Mouth, Clear	Mar 27	Slight
	Moribund	Mar 28	Present
	Death	Mar 29	1.8 days
85D00185	Stain, Mouth, Brown	Mar 27	Moderate
	Twitching	Mar 27	Marked
	Squinting	Mar 27	Slight
	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Death	Mar 28	19.6 h
85D00183	Jumping	Mar 27	Moderate
	Inactive	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Hunched Posture	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Material, Mouth, Clear	Mar 27	Slight
	Death	Mar 28	18.8 h
85D00181	Tremors	Mar 27	Marked
	Jumping	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Squinting	Mar 27	Slight
	Clonic Convulsion	Mar 27	Slight
	Prostrate	Mar 27	Present
	Death	Mar 27	3.6 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00063	Irritable	Feb 16,17	Slight
85D00040	Irritable	Feb 17	Slight
85D00031	Normal	N/A	N/A
85D00019	Normal	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 867 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00276		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate
85D00264		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Marked
	Irritable	Mar 27	Slight
	Material, Perianal, Yellow	Mar 27	Slight
	Twitching	Mar 27	Slight
	Inc. Startle Reflex	Apr 6,7	Slight
85D00259		Hunched Posture	Mar 27
	Marked		
85D00234		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Slight
85D00233		Tonic Convulsion	Mar 27
	Present		
	Inactive	Mar 27,28	Marked
	Tremors	Mar 27	Marked
	Hypertonia	Mar 27	Marked
	Hunched Posture	Mar 27,28	Marked
	Hypotonia	Mar 27,28	Marked
	Material, Perianal, Yellow	Mar 28	Moderate
	Squinting	Mar 27	Slight
85D00230		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 867 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00227		Hunched Posture	Mar 27-29
	Marked		
	Inactive	Mar 27-29	Marked
	Hypotonia	Mar 27-29	Marked
	Tremors	Mar 27	Marked
	Material, Perianal, Yellow	Mar 27-30	Moderate
	Squinting	Mar 27	Slight
	Twitching	Mar 27	Slight
	Hyperactive	Mar 30	Marked
	Inc. Startle Reflex	Mar 31	Moderate
85D00226		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
85D00224		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
	Material, Perianal, Yellow	Mar 27	Slight
85D00222		Hunched Posture	Mar 27
	Marked		
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Twitching	Mar 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 954 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00282	Hunched Posture	Mar 27-30	Marked
	Inactive	Mar 27-30	Marked
	Hypotonia	Mar 27-30	Marked
	Tremors	Mar 27	Marked
	Material, Perianal, Yellow	Mar 28	Moderate
85D00280	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27,29	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
	Twitching	Mar 27	Slight
85D00277	Hunched Posture	Mar 27-31	Marked
	Inactive	Mar 27-31, Apr 3	Marked
	Hypotonia	Mar 27,28	Marked
	Tremors	Mar 27	Moderate
	Material, Perianal, Yellow	Mar 27,28	Moderate
	Irritable	Mar 28-30	Slight
85D00274	Hunched Posture	Mar 27-31	Marked
	Inactive	Mar 27-29	Marked
	Hypotonia	Mar 27-29	Marked
	Tremors	Mar 27	Marked
	Squinting	Mar 27,28	Slight
	Material, Perianal, Brown	Mar 28-31	Marked
	Hyperactive	Mar 30	Moderate
	Inc. Startle Reflex	Apr 6	Moderate
85D00273	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Material, Perianal, Yellow	Mar 27	Slight
	Squinting	Mar 27	Slight
85D00265	Death	Mar 27	3.9 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 954 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00254	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Moderate
85D00249	Prostrate	Mar 27,28	Present
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27,28	Marked
	Tremors	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Material,Mouth, Red	Mar 27,28	Marked
	Squinting	Mar 27	Slight
	Material,Perianal,Yellow	Mar 28	Slight
	Lacrimation	Mar 28	Moderate
	Death	Mar 29	1.9 days
85D00239	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Tremors	Mar 27	Marked
	Material,Perianal,Yellow	Mar 27	Slight
	Material,Nose, Red	Mar 27	Slight
85D000237		Hunched PostureMar 27-	
31,	Marked		
		Apr 3,4	
	Inactive	Mar 27-29	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27-29	Marked
	Squinting	Mar 27	Slight
	Material,Perianal,Yellow	Mar 28-31, Apr 3	Marked
	Stain, Perianal, Orange	Apr 6	Slight
	Irritable	Mar 29,30	Slight
	Hyperactive	Mar 30	Slight
	Inc. Startle Reflex	Apr 3	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1050 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00275	Hunched Posture	Mar 27-30	Marked
	Inactive	Mar 27-30	Marked
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27-29	Marked
	Squinting	Mar 27	Slight
	Twitching	Mar 27	Moderate
	Material,Perianal,Yellow	Mar 28-30	Marked
85D00270	Hunched Posture	Mar 27	Marked
	Tremors	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Marked
	Death	Mar 28	18.4 h
85D00268	Hunched Posture	Mar 27-30	Marked
	Tremors	Mar 27	Marked
	Inactive	Mar 27-30	Marked
	Hypotonia	Mar 27-30	Marked
	Squinting	Mar 28	Moderate
	Material,Perianal,Yellow	Mar 29,30	Moderate
	Death	Mar 31	3.9 days
85D00266	Hunched Posture	Mar 27-31	Marked
	Tremors	Mar 27	Slight
	Inactive	Mar 27-31, Apr 3	Marked
	Hypotonia	Mar 27-29	Marked
	Material,Perianal,Yellow	Mar 28	Slight
85D00261	Hunched Posture	Mar 27	Marked
	Tremors	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Material,Perianal,Yellow	Mar 27,28	Marked
	Twitching	Mar 27	Moderate
	Moribund	Mar 28	Present
	Material,Mouth,Clear	Mar 28	Moderate
	Lacrimation	Mar 28	Moderate
	Death	Mar 29	1.8 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1050 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00258	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
	Hypotonia	Mar 27	Slight
85D00244	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Moderate
	Hypotonia	Mar 27	Moderate
	Tremors	Mar 27	Slight
85D00241	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27,28	Marked
	Hypotonia	Mar 27,28	Marked
	Material,Perianal,Yellow	Mar 27,28	Moderate
	Irritable	Mar 29	Slight
85D00236	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Slight
85D00225	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27,28	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Moderate
	Material,Perianal,Red	Mar 28	Moderate
	Lacrimation	Mar 28	Moderate
	Death	Mar 29	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1160 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00281	Hunched Posture	Mar 27	Moderate
	Irritable	Mar 27	Slight
85D00279	Death	Mar 27	3.5 h
85D00267	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Moderate
	Hypotonia	Mar 27	Marked
85D00257	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Twitching	Mar 27	Marked
	Death	Mar 27	4.7 h
85D00248	Hunched Posture	Mar 27,28	Marked
	Inactive	Mar 27,28	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27,28	Marked
	Material, Eye, Red	Mar 27	Moderate
85D00247	Hunched Posture	Mar 27-31, Apr 3	Marked
	Inactive	Mar 27-31, Apr 4	Marked
	Tremors	Mar 27,28	Marked
	Hypotonia	Mar 27-31, Apr 3	Marked
	Material,Perianal,Yellow	Mar 27-31, Apr 3	Moderate
	Material,Mouth,Red	Mar 29	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1160 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00243	Prostrate	Mar 27	Present
	Tremors	Mar 27	Marked
	Twitching	Mar 27	Marked
	Inactive	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Death	Mar 27	2.8 h
85D00242	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Prostrate	Mar 27	Present
	Twitching	Mar 27	Slight
	Material,Perianal,Yellow	Mar 27	Moderate
	Material,Mouth,Yellow	Mar 27	Slight
	Death	Mar 27	5.8 h
85D00221	Hunched Posture	Mar 27	Marked
	Inactive	Mar 27	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27	Marked
	Prostrate	Mar 27	Present
	Twitching	Mar 27	Marked
	Material, Mouth, Clear	Mar 27	Marked
	Death	Mar 28	19.2 h
85D00229	Hunched Posture	Mar 27-31, Apr 3	Marked
	Inactive	Mar 27-29	Marked
	Tremors	Mar 27	Marked
	Hypotonia	Mar 27-29	Marked
	Twitching	Mar 27	Slight
	Hyperactive	Mar 30	Moderate
	Irritable	Mar 31	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1540 mg/kg TEGDN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00256	Hunched Posture	Apr 4	Marked
	Inactive	Apr 4	Moderate
	Hypotonia	Apr 4	Moderate
	Tremors	Apr 4	Marked
	Twitching	Apr 4	Slight
	Squinting	Apr 4	Slight
	Lacrimation	Apr 4	Moderate
	Death	Apr 5	19.6 h
85D00252	Hunched Posture	Apr 4,5	Marked
	Inactive	Apr 4,5	Marked
	Hypotonia	Apr 4,5	Marked
	Tremors	Apr 4	Moderate
	Twitching	Apr 4	Moderate
	Squinting	Apr 4	Moderate
	Lacrimation	Apr 5	Marked
	Material,Perianal,Yellow	Apr 4,5	Moderate
	Material,Mouth,Red	Apr 5	Marked
	Urine	Apr 5	Present
	Death	Apr 6	1.8 days
85D00250	Hunched Posture	Apr 4	Marked
	Inactive	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Tremors	Apr 4	Marked
	Twitching	Apr 4	Marked
	Death	Apr 5	19.7 h
85D00246	Hunched Posture	Apr 4	Marked
	Inactive	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Tremors	Apr 4	Marked
	Material,Perianal,Yellow	Apr 4	Slight
	Squinting	Apr 4	Slight
	Twitching	Apr 4	Marked
	Death	Apr 5	19.7 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1540 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00245	Hunched Posture	Apr 4	Marked
	Inactive	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Tremors	Apr 4	Marked
	Twitching	Apr 4	Marked
	Material, Mouth, Clear	Apr 4	Moderate
	Squinting	Apr 4	Slight
	Death	Apr 5	20.7 h
85D00238	Prostrate	Apr 4	Present
	Hypotonia	Apr 4	Marked
	Twitching	Apr 4	Marked
	Tremors	Apr 4	Marked
	Death	Apr 4	4.8 h
85D00235	Hunched Posture	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Inactive	Apr 4	Marked
	Twitching	Apr 4	Marked
	Prostrate	Apr 4	Present
	Tremors	Apr 4	Marked
	Squinting	Apr 4	Moderate
	Death	Apr 5	20.8 h
85D00232	Hunched Posture	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Inactive	Apr 4	Marked
	Twitching	Apr 4	Marked
	Squinting	Apr 4	Slight
	Material,Perianal,Yellow	Apr 4	Moderate
	Material,Mouth,Red	Apr.4	Moderate
	Prostrate	Apr 4	Present
	Tremors	Apr 4	Marked
	Death	Apr.5	20.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1540 mg/kg TEGDN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00231	Hunched Posture	Apr 4	Marked
	Inactive	Apr 4	Marked
	Hypotonia	Apr 4,5	Marked
	Twitching	Apr 4	Marked
	Squinting	Apr 4	Moderate
	Tremors	Apr 4	Marked
	Prostrate	Apr 5	Present
	Material, Perianal, Red	Apr 5	Slight
	Lacrimation	Apr 5	Marked
	Death	Apr 6	1.8 days
85D00228	Prostrate	Apr 4	Present
	Twitching	Apr 4	Marked
	Tremors	Apr 4	Marked
	Hypotonia	Apr 4	Marked
	Material, Mouth, Red	Apr 4	Moderate
	Death	Apr 4	4.8 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00093	Normal	N/A	N/A
85D00088	Normal	N/A	N/A
85D00071	Normal	N/A	N/A

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: 931 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00160	149	284	340	354
85D00163	149	268	326	320
85D00176	149	275	337	338
85D00178	152	273	324	315
85D00189	150	263	334	330
85D00192	145	267	342	345
85D00203	141	263	329	328
85D00206	151	267	325	313
85D00210	148	262	323	320
85D00216	142	257	308	292
Mean	147.6	267.9	328.8	325.5
Standard Deviation	3.3	7.7	10.0	17.7
Std. Error of Mean	1.28	2.4	3.2	5.6

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: 1070 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00170	141	270	338	329
85D00175	151	284	336	336
85D00184	150	267	313	311
85D00193	146	280	334	338
85D00196	142	272	342	348
85D00204	149	286	309	311
85D00205	142	242	DEAD	
85D00213	140	276	318	318
85D00214	144	297	385	379
85D00215	139	277	346	333
Mean	144.4	275.1	335.7	333.7
Standard Deviation	4.4	14.5	22.7	21.2
Std. Error of Mean	1.4	4.6	7.6	7.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: 1240 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00161	141	264	311	318
85D00162	137	249	305	395
85D00168	140	255	308	298
85D00171	144	280	DEAD	
85D00174	139	262	301	316
85D00180	153	272	319	331
85D00186	140	253	260	274
85D00188	149	279	351	344
85D00209	142	271	336	332
85D00211	144	268	311	321
Mean	142.9	265.3	311.3	325.4
Standard Deviation	4.9	10.7	25.0	33.2
Std. Error of Mean	1.6	3.4	8.3	11.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: 1430 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00166	142	268	DEAD	
85D00172	152	285	359	335
85D00179	145	261	316	302
85D00182	150	266	DEAD	
85D00198	149	267	DEAD	
85D00202	139	245	DEAD	
85D00207	151	274	DEAD	
85D00219	143	278	DEAD	
85D00220	140	259	DEAD	
Mean	145.7	267.0	337.5	318.5
Standard Deviation	5.0	11.6	30.4	23.0
Std. Error of Mean	1.7	3.9	21.5	16.3

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: 1650 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00181	147	251	DEAD	
85D00183	144	292	DEAD	
85D00185	140	264	DEAD	
85D00190	154	270	DEAD	
85D00199	136	280	DEAD	
85D00200	156	293	DEAD	
85D00201	151	269	DEAD	
85D00218	149	290	DEAD	
Mean	147.1	276.1	N/A	N/A
Standard Deviation	6.9	15.2	N/A	N/A
Std. Error of Mean	2.4	5.4	N/A	N/A

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

MALE: Control

Animal No.	Receipt	Day 2	Day 9	Day 14
85D00019	145	236	313	315
85D00031	130	200	295	305
85D00040	135	220	295	301
85D00063	141	224	293	287
Mean	137.8	220.0	299.0	302.0
Standard Deviation	6.6	15.0	9.4	11.6
Std. Error of Mean	3.3	7.5	4.7	5.8

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: 867 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00222	149	190	239	217
85D00224	161	197	231	221
85D00226	152	208	248	236
85D00227	150	177	201	190
85D00230	150	204	259	242
85D00233	158	196	212	219
85D00234	147	197	238	224
85D00259	150	196	243	226
85D00264	171	218	273	253
85D00276	158	207	267	258
Mean	154.6	199.0	241.1	228.6
Standard Deviation	7.4	11.2	22.7	19.7
Std. Error of Mean	2.3	3.5	7.2	6.2

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: 954 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00237	150	198	223	222
85D00239	141	188	238	223
85D00249	158	222	DEAD	
85D00254	150	177	208	204
85D00265	154	197	DEAD	
85D00273	139	183	224	220
85D00274	149	184	211	207
85D00277	148	192	228	223
85D00280	154	205	252	235
85D00282	153	208	260	257
Mean	149.6	195.4	230.5	223.9
Standard Deviation	5.9	13.6	18.4	16.6
Std. Error of Mean	1.9	4.3	6.5	5.9

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: 1050 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00225	149	186	DEAD	
85D00236	150	185	216	208
85D00241	154	194	228	224
85D00244	155	194	236	220
85D00258	153	191	243	229
85D00261	150	186	DEAD	
85D00266	149	193	236	217
85D00268	152	194	DEAD	
85D00270	151	201	DEAD	
85D00275	151	202	226	230
Mean	151.4	192.6	230.8	222.3
Standard Deviation	2.1	5.9	9.5	9.7
Std. Error of Mean	0.7	1.9	3.9	4.3

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: 1160 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00221	152	209	DEAD	
85D00229	144	171	175	190
85D00242	148	197	DEAD	
85D00243	154	196	DEAD	
85D00247	147	189	210	210
85D00248	163	223	234	246
85D00257	148	195	DEAD	
85D00267	159	211	261	237
85D00279	147	196	DEAD	
85D00281	141	179	228	211
Mean	150.3	196.6	221.6	218.8
Standard Deviation	6.8	15.2	31.8	22.6
Std. Error of Mean	2.2	4.8	14.2	10.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: 1540 mg/kg

Animal No.	Receipt	Dosing	Day 7	Day 14
85D00228	162	207	DEAD	
85D00230	152	196	DEAD	
85D00232	152	196	DEAD	
85D00235	148	204	DEAD	
85D00238	154	203	DEAD	
85D00245	152	200	DEAD	
85D00246	151	203	DEAD	
85D00250	151	216	DEAD	
85D00252	148	248	DEAD	
85D00256	149	216	DEAD	
Mean	151.9	208.9	N/A	N/A
Standard Deviation	4.0	14.9	N/A	N/A
Std. Error of Mean	1.3	4.7	N/A	N/A

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

FEMALE: Control

Animal No.	Receipt	Day 2	Day 9	Day 14
85D00071	140	181	224	215
85D00088	146	180	213	212
85D00093	147	186	234	220
Mean	144.3	182.3	223.7	215.7
Standard Deviation	3.2	3.2	10.5	4.0
Std. Error of Mean	1.4	1.9	6.1	2.3

Appendix G: PATHOLOGY REPORT

GLP Study 84011: MLD Study (Oral)
Principal Investigator: Dr. Gerald F.S. Hiatt

Compound: TEGDN

Species: Rat Strain: Sprague-Dawley Age: 9 weeks

Procedures: Euthanasia: Sodium pentobarbital
Fixative: 10% Buffered formalin
Histopath: Routine

Gross findings (live animals indicated by asterisk *):

MALES/931 mg/kg

LAIR ACC#	ANIMAL ID#	DOSE-DEATH INTERVAL	GROSS OBSERVATIONS
37263*	85D00160	14 days	Not remarkable (NR)
37266*	85D00163	14 days	Right kidney-hydronephrosis, mild
37272*	85D00176	14 days	NR
37273*	85D00178	14 days	NR
37279*	85D00189	14 days	NR
37280*	85D00192	14 days	NR
37283*	85D00203	14 days	NR
37285*	85D00206	14 days	NR
37287*	85D00210	14 days	NR
37292*	85D00216	14 days	NR

MALES/1070 mg/kg

37084	85D00205	6 hrs	Right kidney-hydronephrosis,mild Liver-medial lobe yellow/white foci Liver-other lobes yellow/brown
37268*	85D00170	14 days	NR
37271*	85D00175	14 days	NR
37276*	85D00184	14 days	NR
37281*	85D00193	14 days	NR
37282*	85D00196	14 days	NR
37284*	85D00204	14 days	NR
37289*	85D00213	14 days	NR
37290*	85D00214	14 days	Right kidney-hydronephrosis minimal
37291*	85D00215	14 days	NR

Appendix G (cont.): PATHOLOGY REPORT

MALES/1240 mg/kg

LAIR ACC#	ANIMAL ID#	DOSE-DEATH INTERVAL	GROSS OBSERVATIONS
37090	85D00171	< 1 day	NR
37264*	85D00161	14 days	NR
37265*	85D00162	14 days	NR
37267*	85D00168	14 days	NR
37270*	85D00174	14 days	NR
37275*	85D00180	14 days	NR
37277*	85D00186	14 days	NR
37278*	85D00188	14 days	Right kidney-hydronephrosis, moderate
37286*	85D00209	14 days	NR
37288*	85D00211	14 days	NR

MALES/1430 mg/kg

37077	85D00220	3 hrs	NR
37081	85D00166	4.5 hrs	NR
37085	85D00207	4.5 hrs	NR
37091	85D00182	< 1 day	NR
37094	85D00198	< 1 day	NR
37096	85D00202	< 1 day	NR
37133	85D00219	2 days	Right kidney-hydronephrosis, moderate
37269*	85D00172	14 days	NR
37274*	85D00179	14 days	NR

MALES/1650 mg/kg

37082	85D00181	5.5 hrs	NR
37083	85D00201	6 hrs	NR
37086	85D00218	6 hrs	NR
37092	85D00183	< 1 day	NR
37093	85D00185	< 1 day	NR
37095	85D00200	< 1 day	NR
37118	85D00190	2 days	NR
37119	85D00199	2 days	Liver-diffusely pale red and yellow Right kidney-hydronephrosis, moderate

Appendix G (cont.): PATHOLOGY REPORT

MALES/Vehicle control

LAIR ACC#	ANIMAL ID#	DOSE-DEATH INTERVAL	GROSS OBSERVATIONS
36900*	85D00019	14 days	NR
36901*	85D00031	14 days	NR
36906*	85D00040	14 days	NR
36911*	85D00063	14 days	NR
	FEMALES/867 mg/kg		
37293*	85D00222	14 days	NR
37294*	85D00224	14 days	NR
37295*	85D00226	14 days	NR
37296*	85D00227	14 days	NR
37298*	85D00230	14 days	NR
37299*	85D00233	14 days	NR
37300*	85D00234	14 days	NR
37310*	85D00259	14 days	NR
37311*	85D00264	14 days	NR
37317*	85D00276	14 days	NR
	FEMALES/954 mg/kg		
37078	85D00265	4 hrs	NR
37121	85D00249	2 days	NR
37231*	85D00282	14 days	NR
37302*	85D00237	14 days	NR
37303*	85D00239	14 days	NR
37308*	85D00254	14 days	NR
37314*	85D00273	14 days	NR
37315*	85D00274	14 days	NR
37318*	85D00277	14 days	NR
37319*	85D00280	14 days	NR
	GROUP 5/FEMALES/1050 mg/kg		
37098	85D00270	< 1 day	NR
37120	85D00225	2 days	Liver-diffusely pale red and yellow
37122	85D00261	2 days	NR
37136	85D00268	4 days	NR
37301*	85D00236	14 days	NR
37304*	85D00241	14 days	NR
37305*	85D00244	14 days	NR
37309*	85D00258	14 days	NR
37312*	85D00266	14 days	NR
37316*	85D00275	14 days	NR

Appendix G (cont.): PATHOLOGY REPORT

FEMALES/1160 mg/kg

LAIR ACC#	ANIMAL ID#	DOSE-DEATH INTERVAL	GROSS OBSERVATIONS
37079	85D00279	3.5 hrs	NR
37087	85D00242	5.5 hrs	NR
37088	85D00243	4.5 hrs	NR
37089	85D00257	4.5 hrs	NR
37097	85D00221	< 1 day	NR
37297*	85D00229	14 days	NR
37306*	85D00247	14 days	Right kidney-anterior surface yellow mottled with red wedge-shaped area extending to pelvis with white irregular areas
37307*	85D00248	14 days	NR
37313*	85D00267	14 days	NR
37320*	85D00281	14 days	NR

FEMALES/1540 mg/kg

37153	85D00228	5 hrs	NR
37154	85D00232	< 1 day	NR
37155	85D00235	< 1 day	NR
37156	85D00238	6.5 hrs	NR
37157	85D00245	< 1 day	NR
37158	85D00246	< 1 day	NR
37159	85D00250	< 1 day	NR
37160	85D00256	< 1 day	NR
37161	85D00231	2 days	Liver-diffusely pale & light tan in color Stomach-pinpoint red/black foci in glandular mucosa (petechiae)
37162	85D00252	2 days	Liver-diffusely pale & light tan in color Stomach-multiple pinpoint red/black foci in glandular mucosa (petechiae)

FEMALES/Vehicle control

36915*	85D00071	14 days	NR
36919*	85D00081	14 days	NR
36921*	85D00093	14 days	NR

Appendix G (cont.): PATHOLOGY REPORT

Microscopic findings:

LAIR ACC#	ANIMAL ID#	SEX	DOSE GROUP	FINDINGS
37084	85D00205	M	1070 mg/kg	Liver - Infarcts, severe multifocal (characterized by broad areas of necrotic hepatocytes variably bordered by neutrophils & variably punctuated by hemorrhagic foci) vacuolation centrolobular hepatocytes slight multifocal
37119	85D00199	M	1650 mg/kg	Liver - vacuolation peripportal hepatocytes slight, diffuse congestion slight multifocal
37120	85D00225	F	1050 mg/kg	Liver - congestion slight multifocal vacuolation peripportal hepatocytes very slight diffuse
37161	85D00231	F	1540 mg/kg	Liver - vacuolation peripportal hepatocytes slight multifocal, necrosis of individual hepatocytes peripportal very slight multifocal
37162	85D00252	F	1540 mg/kg	Liver - vacuolation peripportal hepatocytes slight diffuse.

Appendix G (cont.): PATHOLOGY REPORT

LAIR ACC#	ANIMAL ID#	SEX	DOSE GROUP	FINDINGS
37306	85D00247	F	1160 mg/kg	Kidney-mineralization cortex left,very slight focal infarct, severe, focal (characterized by a prominent region of tubular necrosis, peripheral mineralization of cellular debris, moderate tubular epithelial hyperplasia, recanalized vascular thrombi; hemorrhage & congestion) chronic interstitial nephritis moderate,multifocal (characterized by tubular epithelial hyperplasia proteinaceous casts, tubular epithelial necrosis, tubular dilation)

Summary: Thirty-eight animals died before the terminal sacrifice. Infarcts were conspicously present in the liver of one 1070 mg/kg male and the kidney of one 1160 mg/kg female. Hepatocellular vacuolation was present in the livers of one 1070 mg/ml and one 1650 mg/ml male as well as one 1050 mg/ml and two 1540 mg/ml females. The above findings were not considered attributable to TEGDN administration. Hydronephrotic kidneys were considered incidental.

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